Exhibit E

CHAPTER

47

Kidney

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OUTLINE

1. INTRODUCTION

Kidneys play many important and sometimes vital functions to maintain normal homeostasis in the body. The major contributions include excretion of waste products of metabolism, regulation of body water and salt, maintenance of extracellular fluid volume, maintenance of acid–base balance, and elimination of foreign substances such as drugs and chemicals and their breakdown products. Alterations in renal functional capacity can affect any of these functions and have detrimental effects on the body. The kidney is recognized as one of the target organs most vulnerable to the toxic effects of drugs and environmental chemicals. The kidney is particularly susceptible because of the high blood flow to this organ relative to its mass, and the unique property of renal tubular epithelium in concentrating urine and its constituents (e.g., drugs or chemicals) through the countercurrent mechanism. Thus, the kidney is typically exposed to a more localized and a higher concentration of drugs and chemicals than other tissues. In toxicology, refinement of risk assessment requires specific knowledge of the molecular, biochemical, and structural effects of drugs and chemicals at the cellular and subcellular levels. The mechanisms by which nephrotoxicity occurs can be both specific and non-specific in toxicology studies where test agents are used in very large doses; thus, having an understanding of the normal physiology as well as basic tissue responses to diverse toxic

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insults enables the toxicologic pathologist to suggest potential mechanisms of action for nephrotoxicants.

Drug- and chemical-associated renal failure represents a very important cause of disability in humans. Three main clinical entities associated with drug effects on kidney are the nephrotic syndrome, acute renal failure, and chronic renal failure. It is estimated that about 20% of all acute renal failure cases in humans are related to pharmaceutical agents, and that 2–5% of patients admitted to hospital will develop drug-induced acute renal insufficiency. A myriad of therapeutic agents are shown to be injurious to the kidney of humans. For example, cisplatin treatment is associated with nephrotoxicity in up to 50% of treated patients; renal function impairment develops in up to 80% of patients treated with amphotericin B; approximately 50% of hospital-acquired acute renal failures are related to the use of aminoglycosides; and approximately 18% of cases of chronic renal failure have been attributed to the use of analgesics. An estimated 500 000 new patients exposed to drugs on a worldwide basis each year develop end-stage renal disease. The estimated annual costs of dialysis and transplants are substantial, representing a major challenge to the pharmaceutical and chemical industries in developing safer molecules.

In the safety assessment of new molecular entities, the concordance in response to xenobiotics in rat and human strongly supports the rat as a good predictor for human renal hazard. The exceptions in concordance include two categories: immune-mediated drug injury in humans, and the xenobiotic-associated unique alpha2μ-globulin nephropathy syndrome in male rats. The safety assessment based on data from otherwise normal laboratory animals can be challenging compared with the frequently intervening factors in human drug-induced renal injury, such as age-associated variability in renal structure and function, or concomitant conditions affecting renal functions, such as hydration status, hypertension, heart failure, and cirrhosis. The toxic effect exposures are therefore difficult to extrapolate precisely from laboratory animals to humans because of these variable risk factors for renal toxicity.

This chapter emphasizes the structural and biochemical changes that occur in nephrotoxicity and the potential mechanisms involved. The rat is the species of focus, since it is the most important model in renal toxicologic pathology. Gross, microscopic, and ultrastructural anatomy is presented, followed by physiologic considerations, notable species, sex- and age-related differences in susceptibility to nephrotoxicity, and occurrence of common spontaneous renal diseases in laboratory animals. Screening strategies for new chemicals, potential mechanisms of renal injury, and the methods to characterize these mechanisms are reviewed on a subtopographical basis using specific drug and chemical examples for illustration.

2. STRUCTURE, FUNCTION, AND CELL BIOLOGY

Complex interactions between relatively few signaling pathways regulate the multiple steps of renal development. Common signaling pathways that function at multiple stages during kidney development include signaling via Wnts, bone morphogenic proteins (BMPs), fibroblast growth factor (FGF), sonic hedgehog (shh), glial cell derived neurotrophic factor (GDNF)/Ret, and notch pathways. Additionally, transcription factors *WT-1*, *Pax2*, *Odd1*, and *Eyal* play important roles in directing renal development. Major growth factors involved in kidney growth and development are listed in Table 47.1. There are two embryonic kidney precursors, the pronephros and mesonephros; any interruption in their growth could result in renal agenesis. In mammals, the embryological development of the permanent kidney (metanephros) begins when the tip of the mesonephric duct (Wolffian duct), known as the ureteric bud, invades the undifferentiated mesenchyme. The ureteric bud and metanephric mesenchyme induce each other during renal development. The ureteric bud forms branching tubules that eventually differentiate into the collecting ducts and the ureter down to its insertion in the bladder trigone. Concomitantly, the cells of the metanephric mesenchyme differentiate into epithelial cells that will eventually develop into the nephron. Branching tubulogenesis of the ureteric bud is critically important for kidney development. A balance between branching tubulogenesis

TABLE 47.1 Growth Factors and the Kidney

Growth factor	Synthesis sites	Site of action
COX-2	TAL, MD, Papillary interstitial cells	Glomeruli, TAL, MD, renal papilla
Endothelins[a]	Glomeruli, PCT, DCT, CD	Glomeruli, PCT, Henle loops, CD, interstitium
EGF[a]	TAL, DCT	Glomeruli, PCT, DCT, CD
FGFs	Glomeruli	Glomeruli, interstitium
IGFs[a]	Glomeruli, CD	Glomeruli, PCT
HGF[a]	Interstitium	PCT
NGF	CD	Glomeruli, CD
PDGFs[a]	Glomeruli, DCT, CD	Glomeruli, interstitium
TGF-α[a]	TAL, DCT	Glomeruli, PCT, DCT, CD
TGF-β[a]	Glomeruli, PCT, CD	Glomeruli, interstitium
VEGF	Glomeruli, CD	Glomeruli

[a] *Shown to be elevated after nephrectomy.*

Abbreviations: COX-2, cyclooxygenase-2; ETs, endothelins; EGF, epidermal growth factor; FGFs, fibroblast growth factors; IGFs, insulin-like growth factors; HGF, hepatocyte growth factor; NGF, nerve growth ractor; PDGFs, platelet-derived growth factors; TGF-α, transforming growth factor-alpha; TGF-β, transforming growth factor-beta; VEGF, vascular endothelial growth factor; MD, macula densa; PCT, proximal convoluted tubule; DCT, distal convoluted tubule; TAL, thick ascending limb of Henle's loop; CD, collecting duct.

Table modified from Handbook of Toxicologic Pathology, *2nd Ed. W. M. Haschek, C. G. Rousseaux and M. A. Wallig, eds. (2002) Academic Press, Table II, p. 271, with permission.*

with facilitating growth factors such as EGF receptor ligands, HGF, and insulin-like growth factors, and with inhibitory growth factors such as TGF-beta family members, probably regulates these events. Growth factors induce epithelial cell proliferation and migration, and modulate the expression of a variety of proteins. During development, several discrete steps generate the kidney's three-dimensional pattern, including specific branch types, regional differential growth of stems, the specific axes of growth (cortico-medullary, dorso-ventral, and rostro-caudal), and the temporal progression of the pattern.

In developing non-human primate kidneys, Pax2 expression is increased in the condensed mesenchyme surrounding the ureteric bud and in the early renal vesicle and nestin, and WT-1 is diffusely expressed in the metanephric mesenchyme. Podocyte progenitors in the inner cleft of the tail of the S-shaped body express Pax2, nestin, and WT-1 in the early second trimester. With the maturation of the kidney, Pax2 expression is localized to the parietal epithelial cells and WT-1, nestin, and synaptopodin are localized to the podocytes from the mid-third trimester through adulthood. The developing glomerulus is positive for α-smooth muscle actin (α-SMA) and Gremlin (mesangial cells), and CD31 and VEGF (endothelium). These expression patterns suggest possible involvement of the respective signaling pathways and growth factors in the ontogeny of the mammalian kidney.

Development of the human kidney begins as early as the third week of embryonic development, with formation of the pronephros, followed by the mesonephros at 4 weeks' and the metanephros at 5 weeks' gestation. At 9 weeks the first glomeruli appear, and nephrogenesis is complete by 36 weeks' gestation in humans. In mice, the ureteric bud forms from the Wolffian duct at embryonic day 9 (E9), nephron induction begins at E10.5, the first glomeruli appear at E14.5, and nephrogenesis continues after birth for 2 weeks. Timing of completion of nephrogenesis in humans and laboratory animals is summarized in Table 47.2.